

UNIVERSITY OF PENNSYLVANIA

THE NATURE OF PIN-POINT COLONIES
IN RAW AND PASTEURIZED MILK

MARGARET MAY DIEHM

A THESIS

IN BACTERIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

PHILADELPHIA

1929



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41547323_0039

THE NATURE OF PIN-POINT COLONIES IN RAW AND PASTEURIZED MILK

A THESIS

IN BACTERIOLOGY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

MARGARET MAY DIEHM

Reprinted from THE JOURNAL OF INFECTIOUS DISEASES
Vol. 45, No. 1, July, 1929, pp. 61-72

PHILADELPHIA

1929



THE NATURE OF PIN-POINT COLONIES IN RAW AND PASTEURIZED MILK

MARGARET MAY DIEHM

From the School of Hygiene and Public Health, University of Pennsylvania, Philadelphia

The question "what are 'pin-point' colonies?" has puzzled workers in dairies and scientific laboratories since the World War, 1914 to 1918. At that time, the importation of German products for culture mediums was prevented and America had to use domestic products. A condition arose simultaneously in the bacteriologic analysis of milk for a new type of colony appeared at times on the agar plates used in routine analysis. These colonies were very minute and Jacobsen (Fay, p. 350)¹¹ was the first to deal with the problem of the "pin-point colony."

Since the time these colonies were first recognized, numerous workers have tried experimentation with these organisms in milk. The greatest difficulty seemed to be that "pin-point" was such a flexible term and might embrace any colony that was minute. Ayers and Johnson³ define "pin-points" as "colonies that are just visible to the naked eye and appear small under a hand lens." This description may fit one or many types of colonies. Fay¹¹ in advising against the unrestricted use of the term pin-points was the first to give definite measurements and descriptions of the colonies. As a result of his work, he divided the colonies into two types, describing the type by the shape of the colony, viz.:—type A colony was spindle-shaped, measuring 0.150 to 0.175 mm. in its longest diameter. Type B colony was circular, measuring 0.250 to 0.350 mm. in diameter. Furthermore, he separated the organisms he found into three groups: group 1, organisms resembling *Streptococcus lactis*; group 2, chains of 4 to 10 irregular shaped cells, and group 3, long, narrow rods in pairs—one cell being smaller than the other as a daughter cell and measuring 1.95 by 3.70 microns. There seems to be no relationship, however, between the two types of colonies specified and the above three groups.

Other workers in carrying out research on milk and milk products have proposed explanations for the presence of these organisms in milk. The ingredients and the P_H of the culture medium are the most often proposed. These explanations seem logical since it was with the change of ingredients that the

colonies were first recognized. About the same time, the colorimetric method of titration of culture medium began to be utilized in laboratory routine and this was a much more accurate method than any previously employed.

Of the ingredients, the beef extract in the culture medium seems to be the agent which either hinders or encourages the growth of pin-point colonies. Taylor²⁹ in a paper read before the International Association of Dairy and Milk Inspectors states that with the commercial powdered agar, he found pin-point colonies in milk from clean as well as from dirty milk plants, and these colonies did not grow on agar made with Liebig's beef extract. Van Horn³¹ compared counts of more than 40 samples of pasteurized milk and found that with Difco beef extract agar the counts were more than three times as great as those with Liebig's beef extract agar. With raw milk, the counts were slightly higher with Difco beef extract agar. Witte's peptone was used in both cases and the P_H of both lots of mediums was 6.6. Yates and Glover³³ in comparing a pre-war medium with bacto-agar, obtained high counts with the bacto-medium. In all the work done with the different ingredients, the P_H was the same for the lots of medium to be compared.

Typical pin-point colonies do not seem to appear on the more acid medium used routinely in milk analysis. The wide range of P_H of the culture medium allowed by the American Public Health Association¹ is so great (6.2 to 7.0) that this has been studied as a possible cause of pin-point colonies. Yates³⁷ states that the P_H of the medium is responsible for this type of growth and in conjunction with Glover³³ tried agar of different P_H values on the same samples of milk. The agar of P_H 8.1 gave the highest counts in all instances. Cooledge⁷ found that with agar having a P_H of 7.3 he got a count of 317,000 while with a P_H of 6.5 to 6.6 he got a count of 15,400 colonies per cc. on the same sample. Ward³³ found that as the P_H approximates 7.0, conditions are favorable for the appearance of very small colonies which at times lead to very high counts. Faber,⁹ on the contrary, claims that there is no relationship between the P_H and bacterial counts.

Still other workers stated that closely crowded plates (e. g. Ward) tend to produce pin-point colonies. This explanation does not seem correct since this difficulty is easily overcome if the proper dilutions are made. In routine work, experience teaches what the dilutions should be so that there should be no more than 100 to 300 colonies on a plate. Fay³⁰ found by experimentation that this was the range which would allow the least probable error in counting. The standard methods for milk analysis¹ allow 250 colonies as a maximum. This would be 25 colonies in a tenth of the plate and this is by no means crowded. If plates are counted as recommended¹ pin-point colonies will not be missed. According to these standards, plates are to be counted with a magnifying lens of $2\frac{1}{2}$ diameters so that the pin-point colonies will be visible to all workers. When these directions are followed, the counts sometimes are considerably higher than the standard allowed by the United States Public Health Service. The ordinance which they passed and which was later modified by the conference of State and Territorial Health Officers states that grade A milk must not have more than 50,000 colonies per cc., and grade B milk must not have more than 100,000 colonies per cc., from the time of pasteurization until delivery to the consumer.³⁰

In studying the pin-point colonies, Cooledge⁷ states that alkaline organisms were found to thrive as typical pin-point colonies but does not state what a typical colony is. Johnson and Exworthy²¹ claim that pin-point colonies are thermophilic streptococci but do not state the species. Swenarton²⁸ found that 50 out of 52 pin-point colonies were streptococci, 7 of which were similar to *Streptococcus acidominimus*, a species not described by Bergey⁵ but is a new species described by Ayers

and Mudge⁴ as a streptococcus which does not form acid in milk. Ayers and Johnson³ isolated an organism which they called *Lactobacillus thermophilus*. This organism has a thermal death point of 71.1 C. for 30 minutes and therefore, is not killed at pasteurizing temperature. They also state that Rogers isolated an organism resembling *Streptococcus lactis* from a sample of milk which had soured when held at the pasteurizing temperature of 62.8 C. for several hours. Fay,¹¹ and Yates and Glover,³⁸ claim that heat-resisting organisms are responsible for pin-point growth and Dotterer⁸ says that heat resisting organisms cause high bacterial counts in milk. Mudge²⁴ in stating that the standard methods¹ are not standard because of the great variation in the constituents and P_H allowed, also says that typical lactic acid bacteria, mostly streptococci, give rise to pin-point colonies more frequently in pasteurized milk than in raw milk. Breed⁶ claims that *Streptococcus thermophilus* which was found by Robertson as causing pin-points is often mistaken for a heat resistant form of *Streptococcus lactis* but *Str. thermophilus* will not grow below 20 C. and grows best at much higher temperatures.

As an introduction to the study of pin-point colonies it was decided that definite size standards should be determined for the colonies which

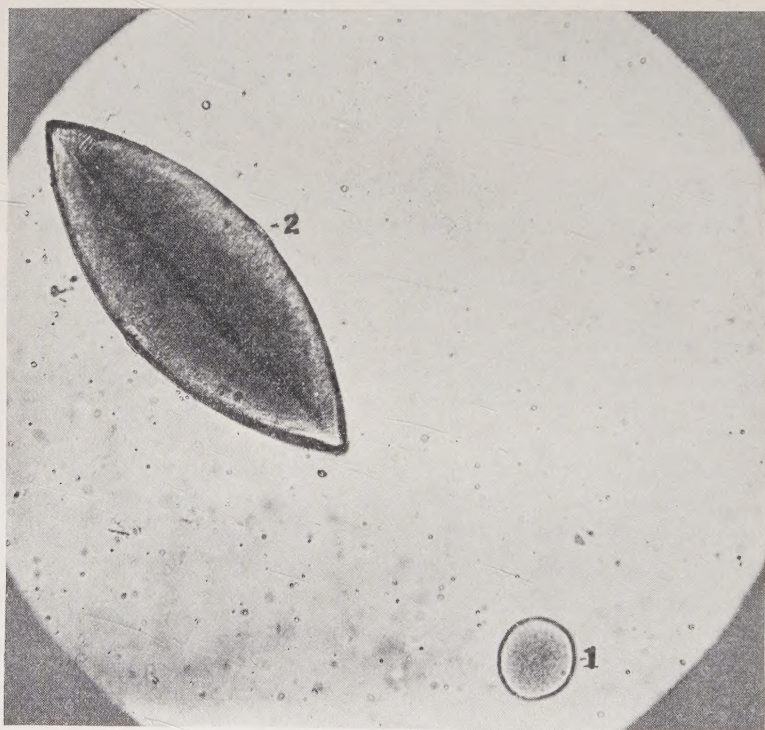
TABLE 1.—MEASUREMENTS OF COLONIES ON AGAR PLATES

Group	Number of Colonies Measured	Shape	Diameters, Mm.	
			Range	Average
a	45	Circular surface	0.10 to 0.16	0.114×0.125
b	25	Circular deep	0.04 to 0.11	0.069×0.079
c	15	Spindle deep	0.07 to 0.09 width 0.11 to 0.16 length	0.085×0.137

were to be isolated and studied. Since macroscopic measurements were impossible because of the minuteness of size, a microscope was calibrated and measurements were made of the colonies on the agar plates which had been made with diluted milk 72 hours or more previously (incubated at 37.5 C. for 48 hours, held at room temperature 24 hours). Table 1 shows the results of the measurements. After completing this phase of the work, it was found that Fay¹¹ had previously measured pin-point colonies, the results of which are given in the first part of this article. By referring to table 1 it will be seen that Fay's colonies were slightly larger and, in addition to the two types he found, there is a deep colony which is decidedly smaller than those studied by Fay. The surface colonies were nearly transparent and the deep colonies were white. All types were finely granular with entire margins. From the measurements given, it will be seen that the colonies will just be visible with the naked eye and will not be much larger with the lens of $2\frac{1}{2}$ diameters recommended by the standard methods.¹

Recognizing the flexibility of the term "pin-points," a phase of this problem was to find a term which would be more suitable and would

conform to the above measurements and descriptions. Minimo-visible, meaning least visible, seems to define the colony and at the same time confine the word to a definite type. When used in this article, it means the colony which is least visible with the naked eye and the smallest in size with a lens of $2\frac{1}{2}$ diameters and conforms to the measurements and descriptions given in table 1. The colonies which conform to these specifications are considered typical and must remain within the size



Minimo-visible (pin-point) colony (1) compared with deep colony of normal milk organism (2). $\times 148$.

range (or approximately) given in table 1, even when transferred to other solid medium and must remain thus for 72 hours. Colonies which may grow larger and white are considered atypical.

As a result of experimental work, it was found that the colonies could be more easily seen if a circle were made on the bottom of the petri dish by means of a wax pencil. A photomicrograph was taken of a deep spindle-shaped colony of an unknown milk organism not of the

minimo-visible group, and of a minimo-visible colony measuring 0.0812 mm. in diameter, with a magnification $\times 148$, to bring out relationship between the two types of colonies. By referring to this plate, it is seen that this type of normal milk organism is more than twice as wide and six times as long as the minimo-visible colony.

In conducting this work, milk samples were studied in which the question of pin-point colonies was not a problem but in which the organisms might be found in small numbers. Samples of milk were collected and plated directly at the plant at hourly intervals from the beginning of the day's operation of the pasteurizing system until the end of the run. After diluting the milk with sterile water to prevent crowding, the samples were plated on Difco beef extract agar made according to the public health methods¹ which without adjustment had a P_H of 6.8. The minimo-visible colonies were not found in large numbers and in no phase of the system did they predominate in the series of tests performed. Two types of pasteurizing systems were tested, one having a continuous flow and the other a pocket-type of holder. Grade B milk had more of this type of colony than grade A, but the total bacterial content of the grade B milk was proportionately higher.

The minimo-visible colonies, after marking in the manner given previously were stroked on to lactose agar plates, by means of a sterile wire loop nicrome needle. For convenience, the bottom of the plate was divided into six sectors by means of a wax pencil, and one colony was stroked over the entire surface of a section. A 1% lactose agar was found to be the most suitable for this work because it contained the normal milk sugar necessary for the growth of normal milk organisms. In transferring the minimo-visible colonies from the original agar plate to the lactose agar plate, because of their minute size, they became imbedded in the agar and the agar was crushed as much as possible to free them. By previous experimentation, this method was found to be more suitable than if the colony were transferred directly to litmus milk. After incubation at 37.5 C., for 24 to 72 hours or longer, some of the organisms grew on the surface of the agar as very minute colonies. These colonies were circular, entire margins, and practically transparent, and were often difficult to see. Such colonies were considered as typical minimo-visible colonies. A slightly larger type of colony which appeared and were white, circular entire margin and more opaque was considered as the atypical type of colony. All colonies which developed on the lactose agar plates were planted into litmus milk and incubated at 37.5 C. for 24 to 168 hours to determine the type of organism responsible for

this growth. Some of the organisms which showed evidence of growth in the litmus milk with the characteristic milk reaction were studied in detail in differential sugar broths and with regard to their heat resistance since most of the cultures were obtained from some phase of the pasteurizing system. The results with cultures of minimo-visible colonies are tabulated. A large number of typical minimo-visible colonies might have

Total number of organisms isolated from minimo-visible colonies.....	1,139
Number of colonies growing typically on lactose agar.....	210
Number of colonies growing atypically on lactose agar.....	63
Number of colonies not growing when transplanted.....	866

ACTION OF ORGANISMS PLANTED IN LITMUS MILK		
Typical		Atypical
120	Acid, coagulation, marked reduction in 24 to 48 hours. Red line of acid reaction becoming deeper with age and the reabsorption of O ₂	25
4	Acid, coagulation, slight reduction.....	0
1	Acid, coagulation.....	0
21	Acid, reduction.....	7
13	Acid.....	6
0	Peptonization.....	1
0	Contracted curd.....	1
51	No change.....	23
57%	% colonies giving typical milk reaction as stated before.....	40%
24%	% organisms not growing when transplanted to litmus milk..	36%

developed the typical *Streptococcus lactis* milk reaction,* if it were not for the following reasons: the drying action of the incubator on the lactose agar may have inactivated some of the cultures; the strain of the organism did not present such a strikingly typical milk reaction, and the heating of the pasteurizing system may have caused the organisms to lose some of the typical reaction. This typical milk reaction according to Wirth³⁶ is sufficient to demonstrate *Streptococcus lactis* (Heim) and differentiates it from other streptococci. Hammer and Baker¹⁶ have separated *Streptococcus lactis* into various varieties and two of these—var. *tardus* and var. *anoxophilus*—correspond to the organisms forming only acid and the slow-reducing types respectively. The morphology of the organisms was practically the same in all cases, spheres in pairs except in old cultures when long chains were evident, gram-positive, measuring 0.5 to 1 micron in diameter and one axis usually slightly longer than the other. The latter fact was especially evident in cultures growing on solid medium.

In transferring the minimo-visible colonies to lactose agar plates a low percentage of growth resulted, probably due to the minuteness of size so that a sufficient number of bacteria could not be transferred with the needle, to the fact that the colony may have been imbedded in the agar, and in the stroke on the new nutritive medium, may not have been

* "Rapid or fairly rapid coagulation, with pronounced reduction. Young cultures entirely reduced except for narrow red band at top, red band increases in width as the culture ages. No gas. No evident digestion, although whey may be expressed." (f. 52.)⁵

freed from the agar, and to the medium which might not have had the requisite constituents favorable for the growth of the organisms.

Heat resistance experiments were performed with cultures isolated and since preliminary experiments pointed to definite facts, the 25 organisms were tested twice. In order to have as nearly the normal surroundings as possible, litmus milk was used as the medium.

A 24 hour litmus milk culture was used for the seeding. One-tenth of a cc. of a thoroughly shaken culture was put into each of twelve tubes, care being taken to avoid touching the side of the tube and thus depositing organisms there which might later be shaken into the milk. The tube was not agitated for the same reason. These tubes were then put into a water-bath the temperature of which was kept constant by means of a thermoregulator. The average temperature was 60 C. with a variation of ± 0.5 degree. Experimentation showed that the milk in the tubes would reach the temperature of the water-bath in about five minutes. At intervals of 15 minutes during the first hour, and at hourly intervals for the subsequent 7 hours, and after 24 hours, a tube was removed from the water-bath and

TABLE 2.—MAXIMUM LENGTH OF TIME ORGANISMS SURVIVED

Source	Number of Cultures Resisting 60 C. for Indicated Period of Time											
	Minutes			Hours								
	Less than 15	30	45	1	2	3	4	5	6	7	24	
Raw milk.....	2	1		1	1							
Pasteurized milk.....			1	2	1	2						
Holder milk.....									1		5	
Cooler milk.....											4	
Bottled milk.....						1			1		2	

put into the incubator. One of the original 12 tubes was kept as a control and was not put into the water-bath. The tubes were examined at 24, 48 and 168 hours. Table 2 shows the heat resistance of the organisms tested and source from which they were obtained:

The cultures did not show any evidence of growth during the time they were held in the water-bath and only grew when they were put in the incubator. Some controls kept at room temperature grew almost as fast as those in the incubator—which showed reduction sometimes within four hours from the time they were put into the incubator.

Normal lactic acid streptococci, on being subjected to higher temperatures (50 to 85 C.) acquire the ability to resist those temperatures. A common belief proposed by Weigmann, Wolff, Trench and Steffen³⁴ as well as Gorini¹⁵ and Holman¹⁸ credits this heat resistance to a casein envelope or a protective albuminous covering around the organism as a result of exposure to high temperatures. From table 2, it is seen that the heat resistance was not tested between the 7 and 24 hours intervals but since the organisms will resist 60 C. for 7 hours, they are capable

of resisting the temperature for the length of time that a continuous-flow pasteurizer would be operated (approximately 8 hours).

Wirth³⁵ divides *Streptococcus lactis* into two groups—group *a* (Heim)—he differentiates by the typical milk reaction above and the organisms of the group are not heat resistant. Meyer and Schönfeld²³ feel that this typical milk reaction is a characteristic of *Streptococcus lactis* but do not separate their organisms (heat resistant and non heat-resistant) according to this milk reaction. Group *b* of Wirth is different in that the milk culture is first red then white and the organisms are heat resistant. It is felt from the observations made during this work that organisms may pass from one group to another because after 24 hours incubation, the cultures which had been subjected to 60 C. for long periods of time showed different stages of reaction in the litmus milk than the same cultures after shorter exposures to that temperature. From the experiment, it was found that the coagulating power of the organism was retarded by the heat, then the reducing ability was affected and finally the acid-producing ability was checked. As an example, in the litmus milk culture that had been subjected for 4 hours to the heat (60 C.)—after incubation for 24 hours, the milk would have an acid reaction, at 48 hours, a soft coagulum would be noticed and finally at 72 hours or more, the typical milk reaction would be evident. Sometimes the first sign of growth would be reduction of the litmus but the first series of conditions were more often present.

The organisms which were tested for heat resistance were also tested in various differential sugar broths, alcohols and gelatin. In previous experiments, litmus milk cultures were used for seeding the tubes but sufficient acid was transferred in this way to obscure the sugar reactions so that a different method was employed. The cultures were stroked on litmus milk agar plates (1 cc. of litmus milk to 10 cc. of cooled melted agar-kept sterile) and after 24 hours incubation, the organisms were removed with a sterile wire loop needle to the sugar broths (1% sugars), a uniform amount being put into each tube. The cultures were incubated and the results determined after 168 hours.

In Orla-Jensen's table (p.131)²⁵ the reaction of *Streptococcus lactis* in the different sugar broths show variations in the different samples of the same species. Variations were encountered in this work with regard to the fermentation of sugars and it is natural to find that cultures do not ferment all the sugars. Two of the strains fermented inulin and some workers might consider them *Streptococcus inulinaceus* but because of other characteristics such as milk reaction and morphology,

it is felt that the cultures were really *Streptococcus lactis* and are comparable to no. 12 in Orla-Jensen's table. A variation in the reactions of the cultures to mannite is not only evident in Orla-Jensen's table but is also recorded in Meyer and Schönfeld's article²³ in which 31.9% of the strains fermented mannitol. About 20% of the cultures isolated and studied in this work fermented mannitol. All the cultures fermented dextrose. A large number of the cultures studied fermented maltose, dextrin and salicin and therefore, they were not *Streptococcus thermophilus* because according to Hucker²⁰ the inability of the organisms to ferment these substances characterises that species. *Streptococcus lactis* is capable of fermenting these sugars.

Hucker¹⁹ states that the fermentation of sucrose is related to the heat resisting ability of the organism and that Orla-Jensen differentiates *Streptococcus thermophilus* from *Streptococcus lactis* in this way; he claims, however, that *Streptococcus thermophilus* grew in milk held at 20 to 30 C. for four hours.²⁰ Hammer and Baker¹⁶ also consider *Streptococcus thermophilus* as the heat resisting type in their classification of *Streptococcus lactis*. However, some of the cultures of *Streptococcus lactis* have the ability to ferment sucrose²⁵ and some cultures of *Streptococcus thermophilus* do not attack this sugar so it seems that the descriptions overlap. In this work, the fermentation of sucrose and the heat resisting ability of the organism could not be correlated. *Streptococcus thermophilus*¹⁹ reduces litmus to a slight extent (p.51).²⁷ Robertson²⁶ states that the thermal resistance alone is not a character of sufficient importance to justify the establishing of a new species and it seems that many of the workers are identifying their cultures with this heat resisting ability as the most prominent characteristic.

Another point which would favor the fact that the organisms studied here were *Streptococcus lactis* and not *Streptococcus thermophilus* is the optimum temperature at which *Streptococcus thermophilus* will grow. The cultures here studied were able to grow fairly rapidly at room temperature while *Streptococcus thermophilus* has an optimum temperature of 40 to 45 C. Orla-Jensen (p.137)²⁵ and Bergey gives 45 C. as the optimum temperature for the same species (p.51).⁵ According to Macfadyen and Blaxall²² the optimum temperature for thermophiles is 60 to 65 C.

Although three of the cultures did not ferment lactose, this would not change the species determination since Orla-Jensen (p.132)²⁵ remarks about strain 12 in his series which lost the ability to ferment lactose. Since the organisms fermented the milk sugar in the milk

originally, they must have lost the ability when transferred to the sugar broths. An alkaline reaction found in the mannitol and raffinose in several instances was either due to mutations of the organisms (tests having been made on organisms which were transferred many times before inoculation into the sugar broths) or the sugars may have been changed in sterilization. Very little alkali was formed. The variations may have been mutations which according to Gorini ^{13, 14} are quite different from the original species.

Fay ¹² in studying pin-point colonies from ice cream found a saccharophilic organism which resembled *Streptococcus lactis* and the organisms when tested for heat resistance, lived through 3 or more pasteurizations without decrease in number. He also found a very small percentage of organisms which would survive 6 hours of continuous heating but does not state if any resisted for a longer period of time. Yates and Glover ³⁸ found pin-point colonies resisted 145 F. for 3½ hours. Breed ⁶ states that during the pasteurization process, nonspore-forming, heat resistant types survive, so Robertson may have been dealing with the same type of organism encountered in this work and not *Streptococcus thermophilus* since that organism is sensitive to low temperatures of cooling and dies before the organisms could be encountered in ice cream or cooler milk.

Since pathogenic streptococci are killed at pasteurizing temperatures ² and commercial pasteurization will free milk from all other pathogens ¹⁷ and no evidence of the pathogenic effect of any strain of *Streptococcus lactis* could be found, the main objection to the cultures would be their resistance to the pasteurizing temperature which would affect the bacterial counts on the milk samples.

CONCLUSIONS

"Minimo-visible" is proposed as more suitable than "pin-point" for those colonies which are just visible to the naked eye, appear small with a hand lens, and have the described characteristics. These colonies are so minute that many are lost in transfer and difficulties arise in cultivating them on artificial culture mediums. Lactose agar (1%) proved the best medium for isolation, and litmus milk for cultivation, of these organisms.

Typical colonies resist pasteurization and are *Streptococcus lactis* with different varieties based on cultural characteristics. The heat resistance is probably due to some protective agency which is acquired in the first heating and subsequent heating does not affect this ability.

In this work the cultures were thermoresistant and not thermophilic because they did not grow at 60 C. and grew only when removed from that temperature to 37.5 C. The organisms isolated from the milk leaving the holder and that leaving the cooler resisted the pasteurizing temperature as long as 7 hours. Heat resistance is not a character of sufficient importance to recognize a new species based on this factor alone. The typical *Streptococcus lactis* milk reaction encountered here is not evident in cultures of *Streptococcus thermophilus*.

The organisms causing minimo-visible colonies are not pathogenic since they are in normal milk, and the interest in them is due mostly to their heat resistance and to the high bacterial count which may be caused by them.

ACKNOWLEDGMENT

Recognition is given here to Dr. Miriam S. Iszard, Assistant Professor of Bacteriology at the University of Pennsylvania for her aid and suggestions throughout the work and to Dr. D. H. Bergey, Professor of Hygiene and Dr. H. F. Smyth, Assistant Professor of Hygiene at the same University, for their helpful suggestions relating to the problem. At the same time, the author wishes to thank Professor Kent for his suggestion bearing on the derivation of a suitable word for the type of colony studied, and the Supple-Wills-Jones and Abbott's Milk Companies for their splendid cooperation in making possible the carrying out of the work with fresh samples.

BIBLIOGRAPHY

1. Standard methods of milk analysis, 1927.
2. Ayers, S. H., and W. T. Johnson, Jr.: Ability of streptococci to survive pasteurization. *J. Agric. Research*, 1914, 2, p. 321.
3. Cause and significance of pin-point colonies from pasteurized milk. *J. Bact.*, 1924, 9, p. 285.
4. The streptococci of the bovine udder. IV. Studies of the streptococci. *J. Infect. Dis.*, 1922, 31, p. 40.
5. Manual of determinative bacteriology, 1925, p. 51.
6. Heat resistant and heat-loving bacteria in their relation to the pasteurization of milk. *New York State Agric. Exper. Sta. Bull.* 559, 1928.
7. A probable explanation of the high counts (pin-point colonies) in pasteurized milk. *Abstr. Bact.*, 1924, 8, p. 20.
8. Some observations on high counts in milk, freshly pasteurized under commercial conditions, 12th Ann. Rept. Intern. Assoc. Dairy and Milk Inspectors, 1923, p. 204.
9. The influence of the pH of agar media upon the bacterial counts of raw and pasteurized milk. *J. Dairy Science*, 1928, 11, p. 401.
10. The probable error of the plate count in the bacteriological examination of milk. *Abstr. Bact.*, 1924, 8, p. 16.
11. Thermotolerant organisms as a cause of so-called pin-point colonies. *J. Bact.*, 1927, 13, p. 347.
12. Thermotolerant and saccharophilic organisms as a cause of pin-point colonies in the bacteriological analysis of ice cream. *J. Bact.*, 1926, 11, p. 96.
13. Mutation de Ferments lactiques par divergences Individuelles. *Abstr. Bact.*, 1922, 6, p. 115.
14. On sudden physiological mutations by individual variations of the lactic acid bacteria. *Abstr. Bact.*, 1922, 6, p. 387.
15. Studies on the biology of lactic acid bacteria: a summary of personal investigations. *J. Bact.*, 1922, 7, p. 271.
16. Classification of the streptococcus lactis group. *Iowa Agric. Exper. Sta. Bull.* 99, 1926.
17. Harding, H. A., and A. R. Ward: The bacterial flora of pasteurized milk. Mathews Industries Inc., Detroit, Mich., and Harding, H. G., Akron Pure Milk Co., Akron, Ohio. *J. Bact.*, 1929, 17, p. 35.
18. The relative longevity of different streptococci. *J. Infect. Dis.*, 1914, 15, p. 295.
19. Certain biochemical reactions produced by the streptococci. *Centralbl. f. Bakteriöl.*, 1, O., 1929, 111, p. 31.
20. Studies on the coccaceae. VII. A study of the cocci resisting pasteurizing temperatures. *New York State Agric. Exper. Sta. Bull.* 134, 1928.

21. Thermophilic streptococci isolated from milk and their relation to pin-point colonies. Abstr. Bact., 1925, 9, (art. 78).
22. Thermophilic bacteria. J. Path. & Bact., 1896, 3, p. 87.
23. Ueber die Unterscheidung des Enterococcus von Streptococcus viridans und die Beziehungen beider zum Streptococcus lactis. Centralbl. f. Bakteriologie, 1, O., 1926, 99, p. 402.
24. Some suggestions regarding the standard methods of milk analysis. Am. J. Public Health, 1927, 17, p. 1034.
25. The lactic acid bacteria. Copenhagen-Hovedkommissionær, 1919.
26. Thermophilic and thermoduric microorganisms with special reference to species isolated from milk. 1. Review of literature. New York State Agric. Exper. Sta. Bull. 130, 1927.
27. Thermophilic and thermoduric microorganisms with special reference to species isolated from milk. 3. Description of non-spore-forming thermoduric organisms isolated from milk. New York State Agric. Exper. Sta. Bull. 131, 1927.
28. Observations on pin-point colony organisms in the Baltimore milk supply. Abstr. Bact., 1925, 8, p. 23.
29. Some observations on the scientific control of milk plants from the standpoint of bacteria. 13th Ann. Rept. Inter. Assoc. Dairy and Milk Inspectors, 1924, p. 287 to 291.
30. U. S. Pub. Health Service Standard Milk Ordinance. Wash. Gov. Printing Office, July 30, 1926, p. 1604.
31. The influence of beef extract on the bacterial counts of raw and pasteurized milk. Abstr. Bact., 1924, 8, p. 16.
32. What ails bacterial counts. A tempest in a teapot. Dairy Products Merchandising-Mathews Industries, Detroit, 1923, 1, no. 7.
33. What ails bacterial counts. Variations in health laboratory methods. Ibid., 1926, 6, no. 6.
34. Ueber das Verhalten der Milchsäurebakterien (Streptococcus lacticus) bei der Dauererhitzung auf 60-63 C. (Modernes Dauerpasteurisierungs Verfahren), Centralbl. f. Bakteriologie, (2), 1916, 45, p. 63.
35. Zur Kenntnis der Streptokokken. Centralbl. f. Bakteriologie, 1, O., 1926, 99, p. 266.
36. Zur Kenntnis der Streptokokken, II. Mitteilung. Ibid., p. 458.
37. Pin-point colonies in plates from pasteurized milk. Abstr. Bact., 1925, 7, no. 24.
38. The pin-point colonies observed in the bacterial examination of milk; their resistance to heat and growth in different culture media, 12th Ann. Rept. Inter. Assoc. Dairy and Milk Inspectors, 1923, p. 252.

